Escola Superior de Biotecnologia
Universidade Católica Portuguesa

FCT

Recovery of Heat-Injured *Listeria innocua*

Fátima A. Miller
Teresa R. S. Brandão
Paula Teixeira
Cristina L. M. Silva

FOODMICRO 2004

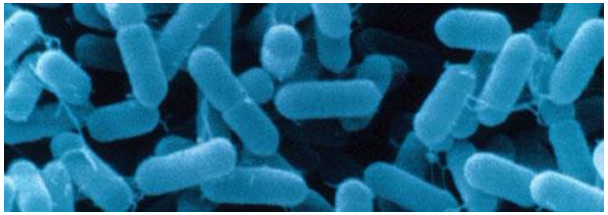
Objectives

- Evaluation of the influence of temperature on thermal inactivation of *Listeria innocua*
- Determination of the incidence of injured cells after thermal stress
- Comparison between two selective media in terms of recovery potential

Context

Listeria monocytogenes

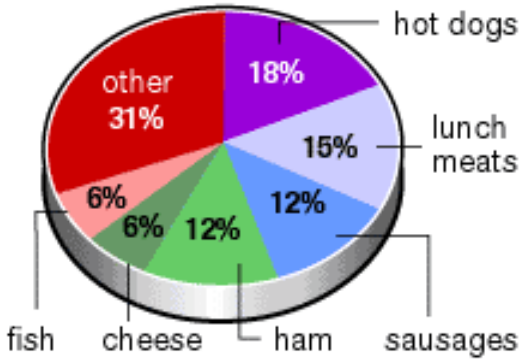
Foodborne pathogen



Widespread distribution

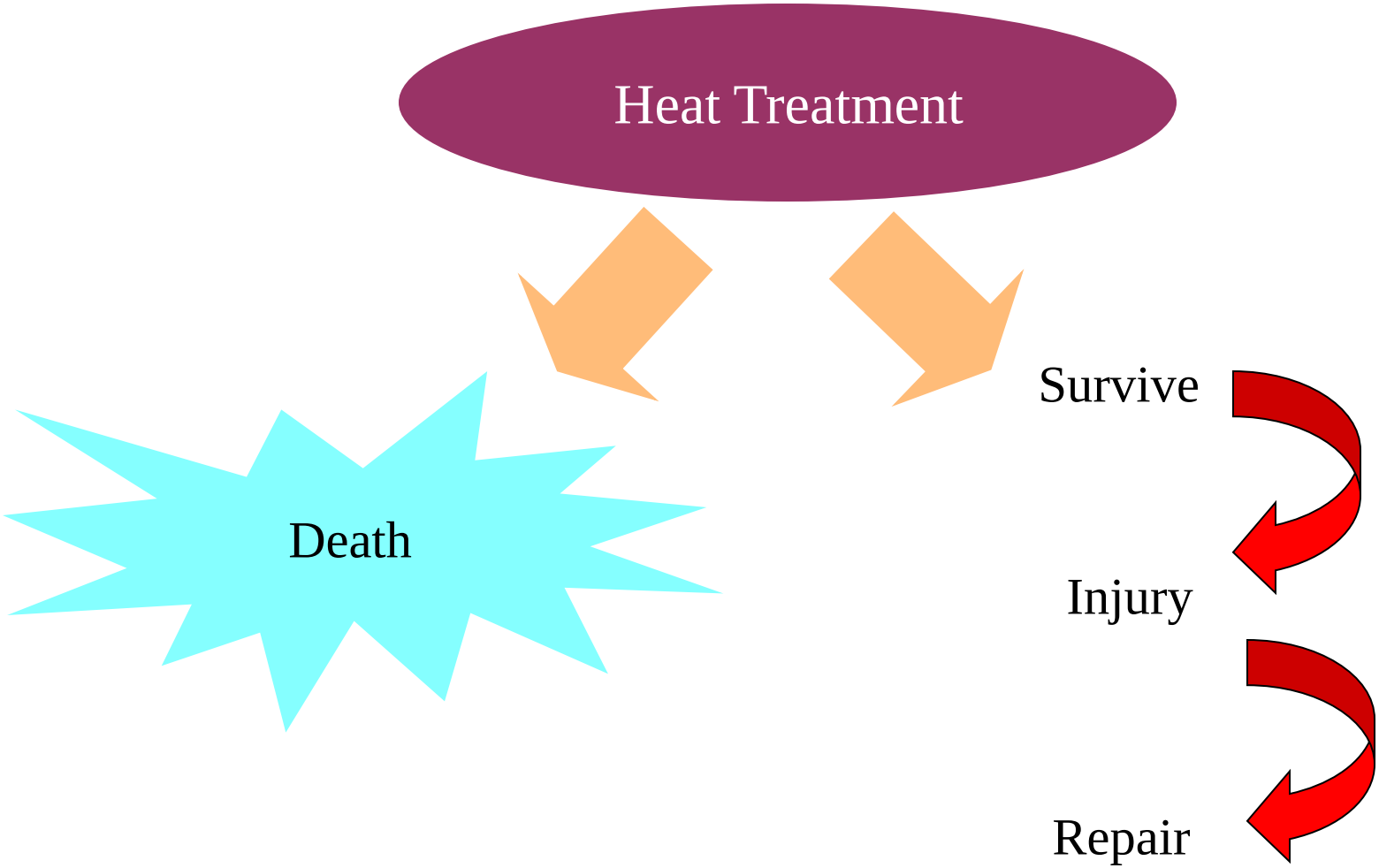
Listeria monocytogenes

Listeria innocua



Foods involved in Listeria outbreaks

Context



Context

Temporary damage of a structure

Loss of a function

- Membrane damage with loss of internal solutes
- Increase sensitivity to unfavourable chemicals

Methodology

Preparation of cultures

L. innocua 10528 at 7ºC

30ºC/24h

30ºC/20h

Stationary phase culture

4000 rpm / 10 min

Ressuspention in TSBYE

Methodology

Heat Treatments

99 ml TSBYE

Agitated water bath

Temperatures (ºC)

52,5 55,0 57,5

60,0 62,5 65,0

Inoculation of 1 ml of cell suspension

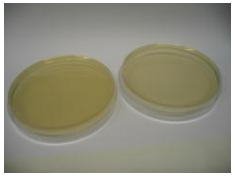
Samples extraction

Methodology

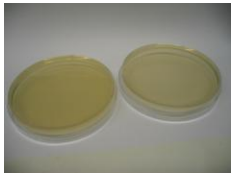
Enumeration

Samples dilution

Non-selective



TSAYE



TSAYE +
5% NaCl

Palcam agar

Selective

Incubation
30ºC / 5 days

Recovery capacity / Degree of injury

$$RIC \equiv \int_0^{total\ process\ time} \log\left(cfu(t)\right)_{selective\ medium}\, dt - \int_0^{total\ process\ time} \log\left(cfu(t)\right)_{TSAYE}\, dt$$

RIC

(Recovery Inhibition Coefficient)

$$\%\ injured\ \ cells \equiv 100 \times \frac{cfu_{TSAYE} - cfu_{selective}}{cfu_{TSAYE}}$$

% Injured Cells

TICC

(Time-averaged Injured Cells Coefficient)

$$TICC \equiv \frac{\int_{t_{initial}}^{t_{final}} \left(\%\ injured\ cells(t)\right) dt}{t_{final} - t_{initial}}$$

Recovery capacity / Degree of injury

Do temperature and medium affect the coefficients?

RIC

(Recovery Inhibition Coefficient)

TICC

(Time-averaged Injured Cells Coefficient)

ANOVA analysis

Results

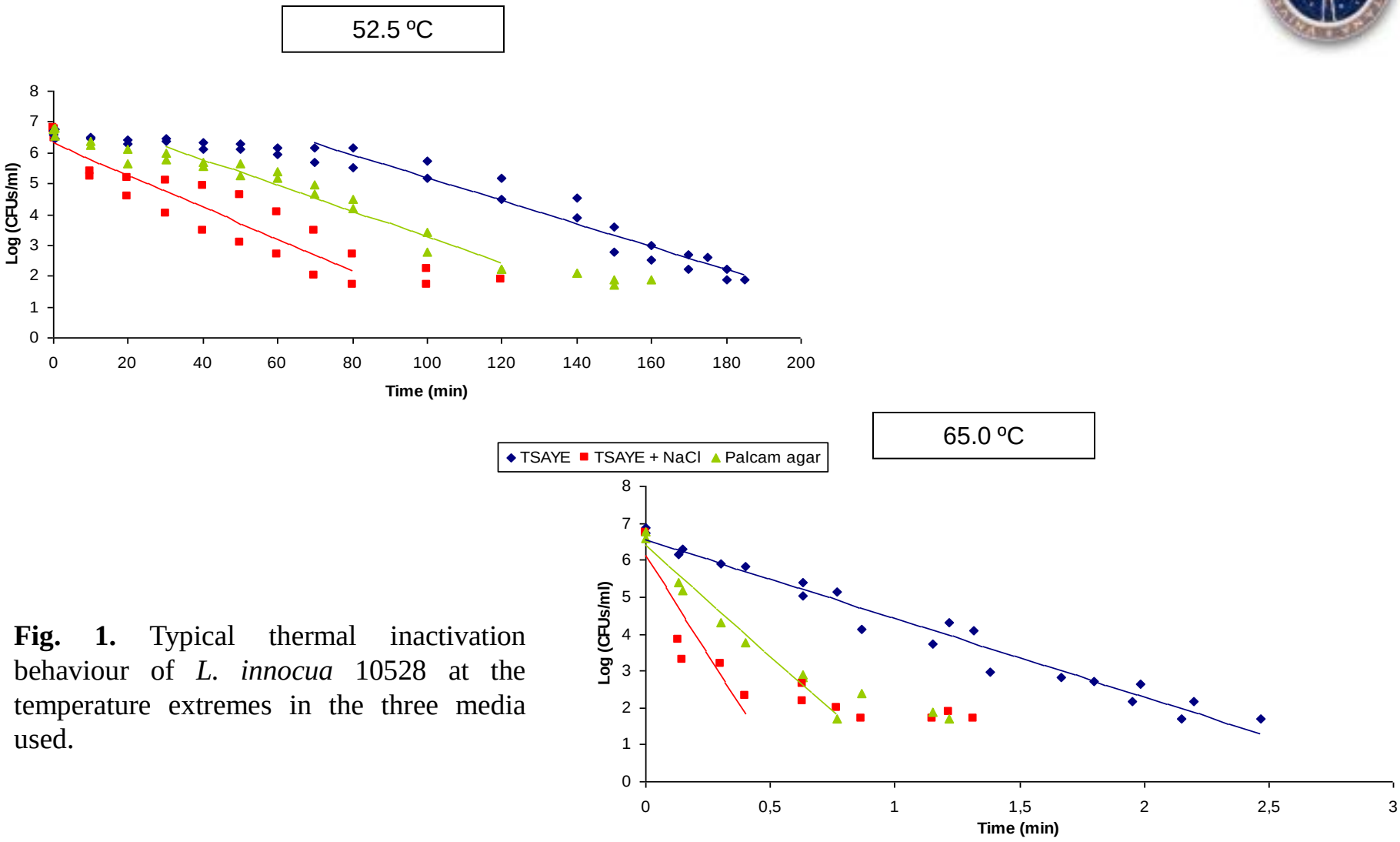


Fig. 1. Typical thermal inactivation behaviour of *L. innocua* 10528 at the temperature extremes in the three media used.

Results

Table 1. Estimated D- and z- values of *L. innocua* 10528 using different growth media

Temperature (ºC)	TSAYE		TSAYE + NaCl		Palcam agar	
	D (min)	R^2 †	D (min)	R^2 †	D (min)	R^2 †
52,5	26,88	0,93	19,31	0,85	23,98	0,95
55,0	16,84	0,89	10,66	0,97	14,37	0,96
57,5	7,65	0,97	3,53	0,95	5,60	0,97
60,0	2,73	0,97	1,08	0,96	1,72	0,94
62,5	0,94	0,98	0,23	0,76	0,46	0,97
65,0	0,47	0,97	0,09	0,82	0,17	0,98
z-value (ºC)	6,73		5,10		5,56	

† Coefficient of determination of the linear regression analysis on survival data

Results

Table 2. Evaluation of the ability of the media to recover injured cells (RIC) and degree of injured cells (TICC) of heat-injured *L. innocua* 10528

Temperature (ºC)	RIC		TICC	
	TSAYE + NaCl	Palcam agar	TSAYE + NaCl	Palcam agar
52,5	-531,12	-279,33	96,47	83,83
55,0	-327,71	-197,70	96,95	82,09
57,5	-120,22	-63,94	97,73	84,39
60,0	-56,11	-39,95	99,19	93,49
62,5	-14,17	-8,89	99,91	94,58
65,0	-6,14	-5,46	99,82	98,96

Discussion

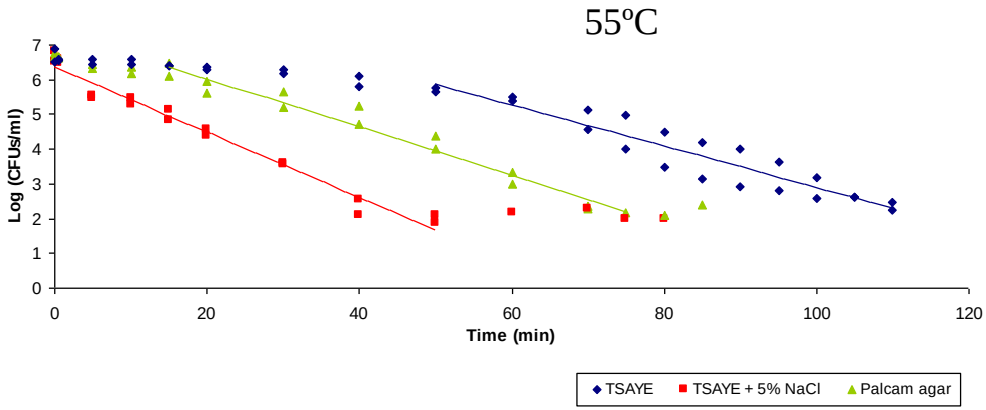
Presence of a Repair Period

For the first hours of incubation, no growth was observed in selective media, particularly in TSAYE + NaCl, but gradually salt tolerance was regained and growth starts;

Gradual loss of resistance of *L. innocua* 10528 with the increase of the heating medium temperature, for all recovery media used;

Discussion

Depending on the temperature and recovery medium used, the overall shape of the survivor curves varied.



$D_{TSAYE} > D_{selective\ media}$

The differences become higher as the temperature increases, being greater from TSAYE + NaCl than from Palcam agar.

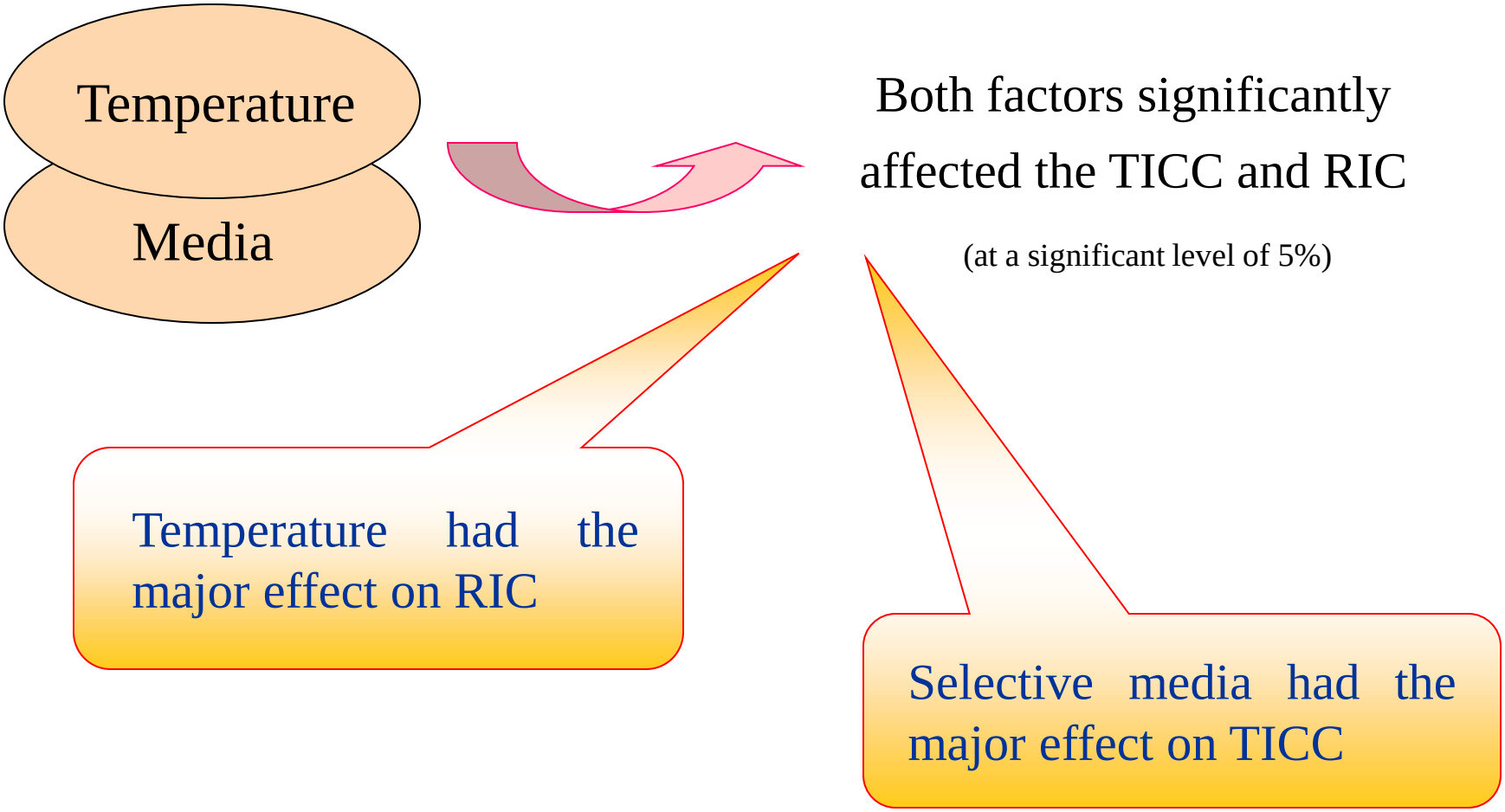
Discussion

Recovery of injured *L. innocua* 10528

TSAYE → Palcam agar → TSAYE + NaCl

Disturbance in cells permeability caused a non-capacity of heat-injured *Listeria* to repair sub-lethal damage in selective media

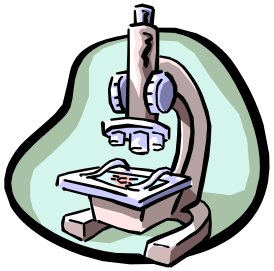
Discussion


Temperature
Media
Both factors significantly affected the TICC and RIC
(at a significant level of 5%)
Temperature had the major effect on RIC
Selective media had the major effect on TICC

Conclusions

Comparing both selective media, Palcam agar is more apropriate for the recovery of injured cells than TSAYE + NaCl medium;

Temperature had a major effect on the recuperation of thermally injured *Listeria*;



When selective media are used to estimate *Listeria* thermal inactivation, the food microbiologist may either fail to detect it or will underreport its numbers.

Final Remarks

To achieve accurate results concerning thermal inactivation of microorganisms, it is crucial the recognition of the presence of potentially injured cells, since sublethal damaged bacteria may recover in food and regain pathogenicity.

It is mainly unacceptable the non-detection of microorganisms that indicate the hygienic quality/safety of a food product. Thus, every attempt should be made to improve the isolation and enumeration procedures to detect all survivors of a thermal treatment.

Thank you for your attention !!!

Acknowledgments

FCT

PhD grant from Fundação para a Ciência e a Tecnologia
SFRH/BD/11358/2002